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in

Testik A. (ed.), Baselga M. (ed.).
2. International Conference on Rabbit Production in Hot Climates

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 41

1999
pages 81-84

VARIABILITY OF CAECAL PARAMETERS IN RABBITS

M.Marounek*, V. Skřivanová and O. Vozna*****

* Institute of Animal Physiology & Genetics, Czech Academy of Sciences, 104 00 Prague 10, Uhříněves, Czech Republic

** Research Institute of Animal Production, 104 00 Prague 10, Uhříněves, Czech Republic

*** Research Institute of Biotechnology in Animal Production, Lvov Veterinary Academy, 290010 Lvov, Ukraine

SUMMARY - Three groups of rabbits of different origin (29, 27 and 28 animals; 3 or 4 - 6 months of age) were slaughtered, their caecal contents analyzed and used for inoculation of *in vitro* cultures. Whereas the caecal pH, dry matter percentages and acetate molar proportions in caecal volatile fatty acids (VFA) were relatively stable, molar proportions of other VFA varied considerably. In *in vitro* incubations, caecal parameters varied somewhat less than *in vivo*. Methane production varied much more than total VFA production. No non-methanogenic rabbit, however, was found. The hydrogen recovery correlated significantly with the methane production and, in two out of three groups of rabbits, also with the propionate molar percentage. The caecal pH was inversely related to VFA concentration.

Key words: Rabbit,caecum, metabolism, variability

RÉSUMÉ *- Des contenus caecaux chez trois groupes des lapins d'origine différente (29, 27 et 28 animaux d'age de 3 a 6 mois) ont été analysé et utilisé pour les incubations in vitro. Les valeurs de pH, pourcentage de la matière sèche (MS) et la proportion molaire d'acide acétique dans le mélange d'acides gras volatiles (AGV) ont été relativement stable dans le contenu de caecum. Les proportions molaires d'autres AGV ont été trouvé plus variable. Pedant des incubations in vitro les paramètres de fermentation étaient moins variable que dans les conditions in vitro. La production du méthane a été trouvé plus variable que la production d'AGV. Néanmois, aucune animal sans méthanogenèse a été observé. Le bilan d'hydrogène était positivement corrélé a la production du méthane et chez deux groupes d'animaux aussi a la proportion molaire du propionate. Les valeurs de pH était negativement corrélé a la concentration d'AGV dans le contenu caecal.*

Mots-clés: *Lapin, caecum, metabolisme, variabilité*

INTRODUCTION

The caecum is the major site of fibre digestion in rabbits. Caecal fermentation converts organic substrates to volatile fatty acids (VFA), gases and compounds incorporated into bacterial cells. The VFA are absorbed, providing an important source of energy for the host (Parker, 1976). The caecum is also supposed to have an important role in the etiology of digestive disturbances. Various nutritional and ontogenic factors affecting the caecal fermentation have been reviewed by Gidenne (1996). According to our knowledge, there is no study on animal-to-animal variation of caecal parameters in rabbits. Age-dependent variation of caecal traits in rabbits has been reported by Piattoni *et al.* (1995). The animal variability should by taken into account when evaluating results of *in vitro* experiments and various *in vivo* measurements. The aim of this study was to assess variability of caecal parameters in three groups of rabbits of different origin.

MATERIAL AND METHODS

Rabbits of the ***first group*** (29 animals) were Hyla 2000 broilers from the Research Institute of Animal Production. Rabbits were fed a granulated concentrate feed containing (%): alfalfa meal (30), wheat bran (30), barley (12), wheat meal (8), extracted soya-bean meal (8), distillers dried grains (8), rapeseed oil (1) and a vitamin-mineral supplement (3). The feed contaned 16.5% crude protein, 13.7% crude fibre and 3.5% fat.

Rabbits of the ***second group*** (27 animals) were Hyla 2000 and Hyplus broilers from a big rabbitry. Animals were fed a granulated feed similar to the feed of the first group. No details on the feed composition were available.

Rabbits of the ***third group*** (28 animals) were crossbred rabbits purchased from small farmers. These rabbits were fed meadow hay, fodder beet and various local forages.

Rabbits of the first and second group were killed at the age of 3 months (live weight: 2.6 - 2.8 kg). Rabbits of the third group were killed at the age of 4 -6 months (live weight: 2.6 - 3.0 kg). Rabbits of the first group were slaughtered *ca* 2h after the last feed intake. Other rabbits were slaughtered after *ca* 14 h fasting. The caeca were emptied by gentle squeezing and used for inoculation of *in vitro* cultures and analyses. The caecal contents (25g) were added to 75 ml of water or McDougall buffer (McDougall, 1949)

with yeast extract (1 g/l) and urea (0.5 g/l). To stop the fermentation in former samples, 0.1 g of $HgCl_2$ was added immediately. The latter samples, i.e. caecal contents diluted with buffer were incubated in 0.3 l serum bottles at 39°C for 24 h. All bottles contained starch, pectin and wheat hemicellulose, 0.5 g each. The bottles were thoroughly flushed with CO_2 and hermetically closed with rubber stoppers. The pH (*ca* 7.2 initially) fell to 5.8 -6.0 in the course of the incubation.

The caecal pH and dry matter were determined immediately. Other analyses were performed using conserved samples. Samples of the headspace gas were taken at the end of the incubation and analysed by gas-liquid chromatography (FID) at the room temperature on a column of the Chromosorb WAW with 15% SP 1220 and 1% H_3PO_4 (Supelco, USA). Total VFA were estimated by titration, after steam distillation. Their molar composition was determined employing the gas chromatograph and the same column at 140 °C. Metabolic hydrogen balance was calculated according to Demeyer (1991) as: $2H_{rec}$ = 100 (2P + 2B + 4M)/(2A + P + 4B), where A, P, B, M represent molar production of acetate, propionate, butyrate and methane, respectively. Such calculation compares the amounts of metabolic hydrogen produced and recovered in reduced end products. The statistical treatment of data was performed using the GraphPAD Software, version 1.14. One-way analysis of variance was used to evaluate differences among groups. Variability of data was expressed as coefficient of variation (CV), i.e. as percentage of standard deviation from the mean.

RESULTS AND DISCUSSION

Results are summarized in Table 1 (*in vivo* measurements) and Table 2 (*in vitro* fermentation pattern). The caecal contents of rabbits slaughtered 2 h after the last feed intake (group 1) had lower pH and higher VFA concentration and dry matter percentage than caecal contents of rabbits slaughtered after 14 - 16 h fasting. In fasted rabbits (groups 2 and 3), molar proportion of caecal acetate was higher and that of propionate and butyrate lower than in non-fasted rabbits. Similar finding was reported earlier by Piattoni *et al.* (1997). Whereas the pH, dry matter contents and acetate percentages were relatively stable (CVs from 2.9 to 10.6%), molar proportions of other VFA varied considerably (CVs from 15.4 to 100%). Caecal parameters tended to be more stable in non-fasted rabbits (group 1) than in rabbits of other groups.

Table 1. Caecal pH, volatile fatty acids (VFA) and dry matter (DM) content in rabbits

Parameter	Rabbit group		
	1	2	3
pH	5.58 ± 0.16[a]	6.26 ± 0.19[b]	6.34 ± 0.30[c]
	(2.9%)	(3.0%)	(4.7%)
Total VFA (µmol/ml)	101.1 ± 20.5[a]	75.0 ± 18.7[b]	74.9 ± 14.8[b]
	(20.3%)	(24.9%)	(19.8%)
Acetate (%)	71.4 ± 2.5[a]	92.1 ± 6.2[b]	84.7 ± 3.3[c]
	(3.5%)	(6.7%)	(3.9%)
Propionate (%)	10.4 ± 1.6[a]	2.7 ± 2.4[b]	6.0 ± 1.8[c]
	(15.4%)	(88.9%)	(30.0%)
Butyrate (%)	16.6 ± 2.5[a]	3.9 ± 3.8[b]	7.6 ± 2.8[c]
	(15.1)	(97.4%)	(36.8%)
Other VFA (%)	1.6 ± 1.6	1.3 ± 1.2	1.7 ± 0.9
	(100%)	(92.3%)	(52.9%)
DM (%)	23.1 ± 1.8[a]	21.7 ± 2.3[a]	19.4 ± 1.5[b]
	(7.8%)	(10.6%)	(7.7%)

Means ± standard deviations. Coefficients of variation are given in parentheses.
[abc] Values in the same row with different letters differ significantly ($P < 0.001$)

Table 2. Production of microbial metabolites in cultures of rabbit caecal contents supplied with starch, hemicellulose and pectin

Parameter	Rabbit group		
	1	2	3
Total VFA (mmol/flask)	11.42 ± 1.26	11.44 ± 1.25	10.32 ± 1.09
	(11.0%)	(10.9%)	(10.6%)
Acetate (%)	72.6 ± 4.9[a]	64.4 ± 3.8[b]	65.6 ± 3.0[b]
	(6.8%)	(5.9%)	(4.6%)
Propionate (%)	8.8 ± 1.5[a]	13.1 ± 3.7[b]	13.0 ± 2.4[b]
	(17.1%)	(28.2%)	(18.5%)
Butyrate (%)	17.0 ± 3.7[a]	20.1 ± 2.8[b]	19.0 ± 3.2[ab]
	(21.8%)	(13.9%)	(16.8%)
Other VFA (%)	1.6 ± 1.6	2.4 ± 1.1	2.4 ± 0.9
	(100%)	(4.6%)	(37.5%)
Methane (µmol/flask)	952 ± 338	1027 ± 507	813 ± 555
	(35.5%)	(49.4%)	(68.3%)
2H recovery (%)	37.1 ± 6.5[a]	45.8 ± 10.4[b]	42.5 ± 10.1[ab]
	(17.5%)	(22.7%)	(23.8%)

Means ± standard deviations. Coefficients of variation are given in parentheses.
[abc] Values in the same row with different letters differ significantly ($P < 0.001$)

In *in vitro* experiments, caecal parameters varied somewhat less than *in vivo*. Methane production varied more than VFA production (CVs 35.5 - 68.3% and 10.6 - 11.0%, respectively). No non-methanogenic rabbit among 84 animals was found. Piattoni *et al.* (1997) observed that in *in vitro* incubations the effect of fasting was not pronounced when substrate was added to cultures. In this study, caecal microbes from non-fasted rabbits (group 1) produced significantly more acetate and less propionate than caecal microorganisms of fasted rabbits (groups 2 and 3). It should be, however, taken into account that diets were not the same in the three groups.

Statistical analysis revealed that the 2H recovery correlated significantly ($P < 0.01$) with the methane production. Correlation coefficients were 0.78, 0.68 and 0.96 in the group 1, 2 and 3, respectively. In groups 1 and 3, the 2H recovery correlated also with the propionate percentage (correlation coefficients: 0.46 and 0.45, respectively). The caecal pH and VFA concentration values were inversely related. Correlation coefficients were -0.61, -0.46 and -0.10 in groups 1, 2 and 3, respectively. The latter coefficient was not statistically significant. Similar relationship between pH and VFA observed Jensen (1977) in the rumen fluid and Piattoni *et al.* (1995) in rabbit caecal conttents. No significant correlation between production of methane and that of other metabolites was found. In the rumen, an inverse relationship between production of methane and propionate exists (Van Nevel *et. al.*, 1974).

Acknowledgement

Financial support was provided by the Grant Agency of the Czech Republic (project No. 524/97/1306).

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